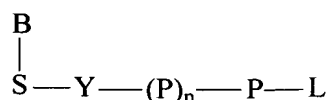


What is claimed is:

1. A method of sequencing a target region of a nucleic acid template,
comprising:
 - 5 a) conducting a nucleic acid polymerization reaction on a solid support,
by forming a reaction mixture, said reaction mixture including a
nucleic acid template, a primer, a nucleic acid polymerizing enzyme,
and one terminal-phosphate-labeled nucleoside polyphosphate selected
from a nucleoside with a natural base or a base analog
10 wherein a component of said reaction mixture or complex of
two or more of said components, is immobilized on said solid
support, and said component or components are selected from
the group consisting of said nucleic acid template, said primer,
and said nucleic acid polymerizing enzyme,
15 and
said reaction results in production of labeled polyphosphate if
said terminal-phosphate-labeled nucleoside polyphosphate
contains a base complementary to the template base at the site
of polymerization;
 - 20 b) subjecting said reaction mixture to a phosphatase treatment, wherein a
detectable species is produced if said labeled polyphosphate is
produced in step a);
 - c) detecting said detectable species;
 - d) continuing said polymerization reaction by adding a different terminal-
25 phosphate-labeled nucleoside polyphosphate selected from the
remaining natural bases or base analogs to said reaction mixture and
repeating steps b and c; and
 - e) identifying said target region sequence from the identity and order of
addition of terminal-phosphate labeled nucleoside polyphosphates
30 resulting in production of said detectable species.
2. The method of claim 1, wherein said nucleic acid template is immobilized on
said solid support in said conducting step.

3. The method of claim 1, wherein said primer is immobilized on said solid support in said conducting step.
4. The method of claim 1, wherein said nucleic acid template and said primer are first hybridized and then immobilized on said solid support in said conducting step.
5. The method of claim 1, wherein said nucleic acid polymerization enzyme is immobilized on said solid support in said conducting step.
6. The method of claim 1, wherein said steps are carried out in a sequential manner in a flow through or a stop-flow system.
7. The method of claim 1, further comprising the step of quantifying said nucleic acid sequence.
8. The method of claim 1, further comprising: quantifying said nucleic acid sequence by comparing spectra produced by said detectable species with a spectra produced from a known standard.
9. The method of claim 1, wherein said nucleic acid polymerizing enzyme is a polymerase.
10. The method of claim 1, wherein said nucleic acid template is an RNA template.
11. The method of claim 1, wherein said nucleic acid template is a DNA template.
12. The method of claim 1, wherein said nucleic acid template is a natural or synthetic oligonucleotide.
13. The method of claim 1, wherein said conducting step and said subjecting step are carried out simultaneously.

14. The method of claim 1, wherein said terminal phosphate-labeled nucleoside polyphosphate comprises four or more phosphate groups in the polyphosphate chain.
- 5 15. The method of claim 1, wherein said detectable species is produced in amounts substantially proportional to the amount of nucleic acid sequence.
16. The method of claim 1, wherein said phosphatase is an acid phosphatase, an alkaline phosphatase or another phosphate transferring enzyme.
- 10 17. The method of claim 1, further comprising including one or more additional detection reagents in said polymerization reaction.
18. The method of claim 17, wherein said one or more additional detection
15 reagents are each independently, capable of a response that is detectably different from each other and from said detectable species.
19. The method of claim 17, wherein one or more of said one or more additional
20 detection reagents is an antibody.
20. The method of claim 1, wherein said detectable species is detectable by a property selected from the group consisting of color, fluorescence emission, chemiluminescence, mass change, reduction/oxidation potential and combinations thereof.
- 25 21. The method of claim 1, wherein said terminal-phosphate-labeled nucleoside polyphosphate is represented by the formula:



wherein

- 30 P is phosphate (PO_3) and derivatives thereof;
- n is 2 or greater;
- Y is an oxygen or sulfur atom;

B is a nitrogen-containing heterocyclic base;

S is an acyclic moiety, carbocyclic moiety or sugar moiety;

P-L is a phosphorylated label which becomes independently detectable when the phosphate is removed,

5 wherein L is an enzyme-activatable label containing a hydroxyl group, a sulfhydryl group or an amino group suitable for forming a phosphate ester, a thioester or a phosphoramidate linkage at the terminal phosphate of a natural or modified nucleotide.

10 22. The method of claim 21, wherein said enzyme-activatable label is selected from the group consisting of chemiluminescent compounds, fluorogenic dyes, chromogenic dyes, mass tags, electrochemical tags and combinations thereof.

15 23. The method of claim 22, wherein said fluorogenic dye is selected from the group consisting of 2-(5'-chloro-2'-phosphoryloxyphenyl)-6-chloro-4-(3H)-quinazolinone, fluorescein diphosphate, fluorescein 3'(6')-O-alkyl-6'(3')-phosphate, 9H-(1,3-dichloro-9,9-dimethylacridin-2-one-7-yl)phosphate, 4-methylumbelliferyl phosphate, resorufin phosphate, 4-trifluoromethylumbelliferyl phosphate, umbelliferyl phosphate, 3-cyanoumbelliferyl phosphate, 9,9-dimethylacridin-2-one-7-yl phosphate, 6,8-difluoro-4-methylumbelliferyl phosphate, and derivatives thereof.

20

25 24. The method of claim 22, wherein said chromogenic dye is selected from the group consisting of 5-bromo-4-chloro-3-indolyl phosphate, 3-indoxyl phosphate, p-nitrophenyl phosphate and derivatives thereof.

25 25. The method of claim 22, wherein said chemiluminescent compound is a phosphatase-activated 1, 2-dioxetane compound.

30 26. The method of claim 25, wherein said 1,2-dioxetane compound is selected from the group consisting of 2-chloro-5-(4-methoxyspiro[1,2-dioxetane-3,2'-(5-chloro-)tricyclo[3,3,1-1^{3,7}]-decan]-1-yl)-1-phenyl phosphate, chloroadamant-2'-ylidenemethoxyphenoxy phosphorylated dioxetane, 3-(2'-

spiroadamantane)-4-methoxy-4-(3''-phosphoryloxy)phenyl-1,2-dioxetane and derivatives thereof.

27. The method of claim 21, wherein said sugar moiety is selected from the group consisting of ribosyl, 2'-deoxyribosyl, 3'-deoxyribosyl, 2', 3'-dideoxyribosyl, 2', 3'-didehydrodideoxyribosyl, 2'-alkoxyribosyl, 2'-azidoribosyl, 2'-aminoribosyl, 2'-fluororibosyl, 2'-mercaptoriboxyl, 2'-alkylthioribosyl, carbocyclic, acyclic and other modified sugars.
28. The method of claim 21, wherein said sugar moiety is selected from ribosyl or 2'-deoxyribosyl sugar.
29. The method of claim 21, wherein said nitrogen-containing heterocyclic base is selected from the group consisting of uracil, thymine, cytosine, 5-methylcytosine, guanine, 7-deazaguanine, hypoxanthine, 7-deazahypoxanthine, adenine, 7-deazaadenine, 2,6-diaminopurine and analogs thereof.
30. The method of claim 1, wherein said target region of a nucleic acid template has a known sequence and wherein the order of addition of terminal-phosphate labeled nucleoside polyphosphates is based on the sequence of the target region.
31. The method of claim 1, wherein said target region of a nucleic acid template has an unknown sequence and wherein the order of addition of terminal-phosphate labeled nucleoside polyphosphates occurs in a preset cycle, said preset cycle being repeated without regard to the identity of the terminal-phosphate labeled nucleoside polyphosphates incorporated in a given cycle.
32. A method of sequencing a target region of a nucleic acid template, comprising:
- a) conducting a nucleic acid polymerization reaction on a solid support, by forming a reaction mixture, said reaction mixture including a nucleic acid template, a primer, a nucleic acid polymerizing enzyme,

and one terminal-phosphate-labeled nucleoside polyphosphate with 4 or more phosphates, selected from a nucleoside with a natural base or a base analog and

5 wherein a component of said reaction mixture or a complex of two or more of said components, is immobilized on said solid support, and said component or components are selected from the group consisting of said nucleic acid template, said primer, and said nucleic acid polymerizing enzyme,

10 and said reaction results in production of labeled polyphosphate if said terminal-phosphate-labeled nucleoside polyphosphate contains a base complementary to the template base at the site of polymerization;

- b) detecting said labeled polyphosphate;
- 15 c) continuing said polymerization reaction by adding a different terminal-phosphate-labeled nucleoside polyphosphate selected from the remaining natural bases or base analogs to said reaction mixture and repeating step b; and
- d) 20 identifying said target region sequence from the identity and order of addition of terminal-phosphate labeled nucleoside polyphosphates resulting in production of said labeled polyphosphates.

33. The method of claim 32, wherein said nucleic acid template is immobilized on said solid support in said conducting step.

25 34. The method of claim 32, wherein said primer is immobilized on said solid support in said conducting step.

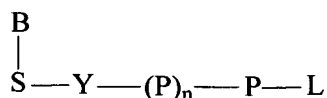
30 35. The method of claim 32, wherein said nucleic acid template and said primer are first hybridized and then immobilized on said solid support in said conducting step.

36. The method of claim 32, wherein said nucleic acid polymerization enzyme is immobilized on said solid support in said conducting step.

37. The method of claim 32, wherein said steps are carried out in a sequential manner in a flow through or a stop-flow system.
- 5 38. The method of claim 32, further comprising the step of quantifying said nucleic acid sequence.
39. The method of claim 32, further comprising: quantifying said nucleic acid sequence by comparing spectra produced by said detectable species with a spectra produced from a known standard.
- 10
40. The method of claim 32, wherein said nucleic acid polymerizing enzyme is a polymerase.
- 15 41. The method of claim 32, wherein said nucleic acid template is an RNA template.
42. The method of claim 32, wherein said nucleic acid template is a DNA template.
- 20
43. The method of claim 32, wherein said nucleic acid template is a natural or synthetic oligonucleotide.
44. The method of claim 32, further comprising including one or more additional detection reagents in said polymerization reaction.
- 25
45. The method of claim 44, wherein said one or more additional detection reagents are each independently, capable of a response that is detectably different from each other and from the said labeled polyphosphate.
- 30
46. The method of claim 44, wherein one or more of said one or more additional detection reagents is an antibody.

47. The method of claim 32, wherein said labeled polyphosphate is detectable by a property selected from the group consisting of color, fluorescence emission, mass change, reduction/oxidation potential and combinations thereof.

5 48. The method of claim 32, wherein said terminal-phosphate-labeled nucleoside polyphosphate is represented by the formula:



wherein

P is phosphate (PO₃) and derivatives thereof;

10 n is 3 or greater;

Y is an oxygen or sulfur atom;

B is a nitrogen-containing heterocyclic base;

S is an acyclic moiety, carbocyclic moiety or sugar moiety; and

P-L is a phosphorylated label,

15 wherein L is a label containing a hydroxyl group, a haloalkyl group, a sulfhydryl group or an amino group suitable for forming a phosphate ester, a phosphonate, a thioester or a phosphoramidate linkage at the terminal phosphate of a natural or modified nucleotide.

20 49. The method of claim 48, wherein said label is selected from the group consisting of fluorescent dyes, colored dyes, mass tags, electrochemical tags and combinations thereof.

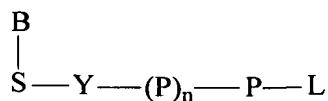
25 50. The method of claim 49, wherein said fluorescent dye is selected from the group consisting of a xanthene dye, a cyanine dye, a merrocyanine dye, an azo dye, a porphyrin dye, a coumarin dye, a bodipy dye and derivatives thereof.

30 51. The method of claim 49, wherein said colored dye is selected from the group consisting of an azo dye, a merrocyanine, a cyanine dye, a xanthene dye, a porphyrin dye, a coumarin dye, a bodipy dye and derivatives thereof.

52. The method of claim 48, wherein said sugar moiety is selected from the group consisting of ribosyl, 2'-deoxyribosyl, 3'-deoxyribosyl, 2', 3'-dideoxyribosyl, 2', 3'-didehydrodideoxyribosyl, 2'-alkoxyribosyl, 2'-azidoribosyl, 2'-aminoribosyl, 2'-fluororibosyl, 2'-mercaptoriboxyl, 2'-alkylthioribosyl, carbocyclic, acyclic and other modified sugars.
53. The method of claim 48, wherein said sugar moiety is selected from ribosyl or 2'-deoxyribosyl sugar.
54. The method of claim 48, wherein said nitrogen-containing heterocyclic base is selected from the group consisting of uracil, thymine, cytosine, 5-methylcytosine, guanine, 7-deazaguanine, hypoxanthine, 7-deazahypoxanthine, adenine, 7-deazaadenine, 2,6-diaminopurine and analogs thereof.
55. The method of claim 32, wherein said target region of a nucleic acid template has a known sequence and wherein the order of addition of terminal-phosphate labeled nucleoside polyphosphates is based on the sequence of the target region.
56. The method of claim 32, wherein said target region of a nucleic acid template has an unknown sequence and wherein the order of addition of terminal-phosphate labeled nucleoside polyphosphates occurs in a preset cycle, said preset cycle being repeated without regard to the identity of the terminal-phosphate labeled nucleoside polyphosphates incorporated in a given cycle.

57. A nucleic acid detection kit comprising:

- a) at least one terminal-phosphate-labeled nucleoside polyphosphate according to the formula:

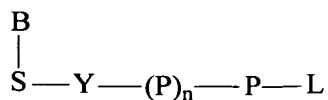


wherein

P=phosphate (PO₃) and derivatives thereof;

- n is 2 or greater;
 Y is an oxygen or sulfur atom;
 B is a nitrogen-containing heterocyclic base;
 S is an acyclic moiety, carbocyclic moiety or sugar moiety;
 5 P-L is a phosphorylated label which becomes independently detectable when the phosphate is removed,
 wherein L is an enzyme-activatable label containing a hydroxyl group, a sulfhydryl group or an amino group suitable for forming a phosphate ester, a thioester or a phosphoramidate
 10 linkage at the terminal phosphate of a natural or modified nucleotide;
- b) at least one enzyme is selected from the group consisting of DNA polymerase, RNA polymerase and reverse transcriptase; and
 c) a phosphatase.
- 15
58. The kit of claim 57, wherein said terminal-phosphate-labeled nucleoside polyphosphate comprises four or more phosphate groups in the polyphosphate chain.
- 20 59. The kit of claim 57, wherein said sugar moiety is selected from ribosyl or 2'-deoxyribosyl sugars.
60. The kit of claim 57, wherein said nitrogen-containing heterocyclic base is selected from the group consisting of uracil, thymine, cytosine, 5-methylcytosine, guanine, 7-deazaguanine, hypoxanthine, 7-
 25 deazahypoxanthine, adenine, 7-deazaadenine, 2,6-diaminopurine and analogs thereof.
61. The kit of claim 57, wherein said enzyme-activatable label is selected from the group consisting of chemiluminescent compounds, fluorogenic dyes,
 30 chromogenic dyes, mass tags, electrochemical tags and combinations thereof.
62. A nucleic acid detection kit comprising:

- a) at least one terminal-phosphate-labeled nucleoside polyphosphate according to the formula:



wherein

5

P=phosphate (PO₃) and derivatives thereof;

n is 3 or greater;

Y is an oxygen or sulfur atom;

B is a nitrogen-containing heterocyclic base;

S is an acyclic moiety, carbocyclic moiety or sugar moiety;

10

P-L is a phosphorylated label,

wherein L is a label containing a hydroxyl group, a haloalkyl group, a sulfhydryl group or an amino group suitable for forming a phosphate ester, a phosphonate, a thioester or a phosphoramidate linkage at the terminal phosphate of a natural or modified nucleotide;

15

and

- b) at least one enzyme is selected from the group consisting of DNA polymerase, RNA polymerase and reverse transcriptase.

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